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Effectiveness of Myoinositol alone or in Companion with Metformin in Improving Hormonal, Metabolic, and Clinical Features of PCOS Women [#]

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Abstract

Polycystic ovary syndrome (PCOS) referring to a syndrome that is recognized as a life-course disease and has both metabolic and reproductive signs; main pathophysiological cause includes insulin resistance, hyperandrogenism, and oxidative stress state. The study aimed to assess the impact of Myoinositol alone, metformin alone, or myoinositol in companion with Metformin, on improving clinical, metabolic, and hormonal parameters in females with PCOS. A clinical trial that was prospective, randomized, and comparative on 54 female patients with PCOS (aged 18-40 y) are divided into three groups: group1 patients allocated to receive Myo-inositol(4g), group2 patients assigned to receive Metformin(1g) and group3 patients assigned to receive Myo-inositol(4g) + Metformin(1g) all for three months. Baseline and post-intervention fasting blood samples were collected to evaluate hormonal {testosterone, luteinizing hormone, follicle stimulating hormone and estradiol}, metabolic parameters {fasting insulin, fasting glucose}, and oxidative stress biomarkers{Glutathione peroxidase}. Metformin and Myoinositol together lead to a significant drop free testosterone (P = 0.002), LH (**P=** 0.003), LH to FSH ratio(P=0.004), FSI (P= 0.012), the Homeostatic Model Assessment of insulin resistance (HOMA IR) HOMA IR (p= 0.019), hirsutism score (p<0.001) and acne score (p= 0.003), besides substantial increase in GPX (P =0.02). Meanwhile, Myoinositol supplement causes a substantial drop in free testosterone (p=0.013), FSI (P =0.02), and HOMA – IR (P value=0.025) also a substantial rise in Glutathione peroxidase (GPX) (P=0.005). Combining Myoinositol with metformin resulted in improving clinical, metabolic, hormonal parameters, as well as oxidative indicators for PCOS females.

Key words: Polycystic Ovary Syndrome, Myoinositol, Metformin, Hormones, HOMA-IR.

فعالية الميواينوزيتول مقارنة بالميتفورمين في تحسين الخصائص الهرمونية والايضية والسريرية لنساء متلازمة تكيس المبايض[#]

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الخلاصة

متلازمة تكيس المبايض تشير الى انها متلازمة يتم التعرف عليها على أنها مرض يستمر مدى الحياة وله علامات ايضية وانجابية؛ يشمل السبب الفيزيولوجي المرضي الرئيسي مقاومة الأنسولين وفرط الأندروجين وحالة الإجهاد التأكسدي. هدفت الدراسة إلى تقييم تأثير الميواينوزيتول بمفرده أو مع الميتفورمين، على تحسين المعايير السريرية، والايضية، والهرمونية للإناث المصابات بمتلازمة تكيس المبايض. تم تقسيم التجربة السريرية التي كانت مستقبلية وعشوائية ومقارنة على ٥٤ مريضًا (تتراوح أعمارهن بين ١٨ و٤٠ عامًا) إلى ثلاث مجموعات: المجموعة ١ المخصصة لتلقي ميواينوزيتول (٤ جم) والمجموعة ٢ المخصصة لتلقي الميتفورمين (١ جم) والمجموعة ٣ المخصصة لتلقي ميواينوزيتول (٤ جم) + الميتفورمين (١ جم) لمدة ثلاثة أشهر. تم جمع عينات الدم للمرضى بعد الصيام (٨-١٠ ساعات) قبل وبعد ثلاث أشهر من العلاج لتقييم العوامل الهرمونية والايضية والمؤشرات الحيوية للتوتر التأكسدي. يؤدي الميتفورمين والميواينوزيتول معًا إلى انخفاض كبير في هرمون التستوستيرون الخالي من الانخفاض (P = 0.002) ، (P 0.003) LH، نسبة LH إلى FSH (P = 0.004) ، (P= 0.012) FSI، مقاومة الانسولين p (0.019=، (p <0.001)درجة الشعرانية ودرجة حب الشباب (p = 0.003) ، إلى جانب زيادة كبيرة في .GPX (P= 0.02) وفي الوقت نفسه، يتسبب مكمل الميواينوزيتول في انخفاض كبير في هرمون التستوستيرون الحر(P = 0.013) ، و(P 0.02) FSI، (P = 0.025) وقيمة مقاومة الانسولين أيضًا ارتفاعًا كبيرًا في GPX (P = 0.005). استخدام عقار الميتفورمين والميواينوزيتول يؤدي إلى تحسين المعايير السريرية والايضية والهرمونية، فضلاً عن المؤشرات المؤكسدة لإناث متلازمة تكيس المبايض.

الكلمات المفتاحية: متلازمة تكيس المبايض، الميواينوزيتول، الميتفورمين، الهرمونات، مقاومة الانسولين.

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Introduction

Polycystic ovarian syndrome is a heterogeneous illness with various signs and symptoms as well as a difficult diagnostic that differs between countries depending on the guidelines used, such as the National Institutes of Health (NIH) standards, the Androgen Excess Society (AES) standards, and the Rotterdam 2003 standards[1].PCOS was considered as the most prevalent endocrine condition which can influence up to 20% of females of reproductive age[2],Considering the Rotterdam criteria, clinical and/or biochemical hyperandrogenism, polycystic ovaries, and oligo- or anovulation—two of the three important aspects —must be present in women for PCOS to be diagnosed[3] . The pathogenesis of this syndrome is substantially impacted by insulin resistance (IR) and compensatory hyperinsulinemia [4], which increase Luteinizing hormone (LH) impact on ovarian androgen synthesis. Furthermore the inhibition of sex hormone binding globulin (SHBG) production by androgens and insulin raises free androgen levels and exacerbates clinical androgen excess [5].These women have a greater chance of getting type 2 diabetes, hypertension, dyslipidaemia, cancer, also CVDs as a result of IR[6].

According to the HOMA-IR formula, a range of (0.5 -1.4) is considered good. Insulin sensitivity is defined as less than 1.0, early insulin resistance as more than 1.9, and considerable insulin resistance as more than 2.9[7].

The first medication to increase insulin sensitivity was metformin, used in PCOS to investigate how insulin resistance contributes to the pathophysiology of the disorder[8]. Although the exact mechanism of action is still unknown, metformin is known to decrease gastrointestinal glucose absorption[9], reducing hepatic glucose synthesis , mostly via mildly and momentarily inhibiting the mitochondrial respiratory chain complex[10].In addition, it causes a reduction of fasting plasma insulin level and improvement of insulin sensitivity[11] and results in weight loss[12]. In addition, the antiandrogenic effects[13],which occur by limiting the production of androgen in the adrenal gland and ovary, lowering pituitary luteinizing hormone and raising sex hormone binding globulin (SHBG) synthesis in the liver[8] so, this effect considerably lessened the hirsutism symptoms.

Myoinositol, an insulin-sensitizing substance, cyclic carbohydrate with six hydroxyl groups is referred to as inositol (cyclo- hexanehexol) [14]. For the treatment of PCOS, myoinositol is one of the most interesting compounds being researched[15].It serves many distinct functions in numerous organisms including: controlling metabolic flow, phosphate levels, and ion channel permeability insulin signalling, embryonic development, and stress response[16].

Myo-inositol enters cells as Phosphatidyl Myoinositol is transformed into inositol-triphosphate, which serves as a second messenger for insulin, thyroid stimulating hormone (TSH), and follicle-stimulating hormone that is intracellular[14].Given its known ability to increase insulin sensitivity, Myoinositol has been used to prevent and treat a variety of metabolic conditions linked to IR, including metabolic syndrome, gestational diabetes mellitus, and polycystic ovarian syndrome (PCOS)[17].

Numerous studies have shown that using Myoinositol as a dietary supplement helps PCOS women's metabolic and hormonal characteristics but, due to the limited number of participants enrolled, these researches were limited and need further research. The majority of these research' findings suggest that combining myoinositol and metformin lowers insulin resistance and normalizes hormonal imbalances [18, 19, 20], while some studies found improved menstruation and a rise in the number of pregnancies [21,22]. According to the results of these research, myoinositol and metformin together were the most effective treatments for PCOS women, no study used such combination among Iraqi PCOS women.

Several clinical studies were reported exploring the role of myoinositol supplement in combination with metformin, but to the best search, no study used such combination among Iraqi PCOS women. Consequently, the purpose of the current investigation was to assess clinically if Myoinositol, metformin, or combination can improve hormonal, metabolic, and oxidative markers.

This research focuses on restoring hormonal balance and oxidative status, consequently enhance fertility and lower risk of T2DM and cardio-vascular disease. The first insulin-sensitizing medication used in the treatment is metformin, but due to its side effects, a recent study focused on the use of another more desirable and favorable drug, myoinositol, which is the preferred option. This study aimed to explore the effectiveness of myoinositol alone or in companion with metformin in improving PCOS clinical and biochemical features.

Design of the study

This research was open label, randomized (simple randomization) and prospective, comparative clinical research to explore the effectiveness of adding a myo-inositol supplement to the standard treatment of PCOS in both married and unmarried women. The primary outcome was assessed objectively by measuring hormonal, metabolic, and antioxidant parameters. The second outcome was assessed subjectively through the improvement of the patient's symptoms. Three groups of eligible patients were assigned: 18 PCOS patients in **Group 1** treated with Myo-inositol 4g once daily after a dinner for three months. **Group 2**:

consists of 18 PCOS patients receiving Metformin 500 mg (once daily for two weeks then, two times daily) after meals for three months. **Group 3:** include 18 PCOS patients treated with Myo-inositol 4g once daily after meal plus Metformin (500 mg once daily for two weeks, then two times daily) after meals for three months period. Before starting the intervention and after 3 months, 5mL blood sample was withdrawn from patients during a day 2-3 of the cycle for hormonal evaluation and other investigations.

Inclusion Criteria

Women in reproductive age range (18-40 yrs.)

Women who meet the Rotterdam criteria[23] for PCOS, which include bio-chemical, clinical, hyperandrogenism, oligo or anovulation, PCOM (Poly cystic ovarian morphology), or who have an ovarian volume larger than 10 ml. Women with PCOS of reproductive age and willing to become pregnant.

Both married and unmarried women were enrolled.

Patients with PCOS who visited the clinic complaining of menstrual irregularities like oligomenorrhea for more than a month were treated with progesterone ampules 50 mg two ampules once in order to regulate their menstrual cycles. These patients were then enrolled in the study following hormonal testing in the 2-3 day of the menstrual cycle.

All participants were advised to not use any medicine while the experiment was running unless they had talked to the doctor first to prevent effect of other agents.

Exclusion criteria

PCOS women with other endocrine disorders (hypothalamic, pituitary, and adrenal disturbances, hypo- or hyperthyroidism, Pregnancy, and nursing women, also women with bleeding due to any pathological condition, such as a fibroids, polyps, or cervical disease and women taking supplements and drugs other than the study intervention are not allowed to participate in the study.

Patients and Methods

The study included a total of 54 participants with PCOS through their visits to the public hospital of Samarra general hospital and the specialized clinic; Only 49 patients between the ages of 18 and 40 completed the study due to 2 patients become pregnant while 3 patients refuse to continue treatment. PCOS patients were managed by a gynaecologist, diagnosed using Rotterdam criteria[23] and treated in agreement with the highest standards of medical care.

Laboratory investigations

About 1ml of serum is used directly for testing fasting serum insulin (FSI), Free testosterone (FT), Follicular- stimulating hormone (FSH), luteinizing- hormone (LH), Estradiol (E2), and high sensitivity C-reactive protein(hsCRP). Since these tests should be done within less than one month of sample collection and some for-diagnosis purposes, The residual serum samples were kept in an Eppendorf tube and kept at (-40 ^{0}C) until subsequent tests were run like Glutathione peroxidase (GPX).Also, Waist circumference (WC) was measured using a measuring tape at the minimum the distance between the lateral costal edge and the iliac crest and BMI for patients was calculated from their weight and height utilizing the following equation [24]**:** BMI= weight (kg) / [height (m)]^2

Calculation of insulin resistance using a homeostatic model (HOMA-IR) for patients use their fasting serum insulin in mIU/ml and fasting blood glucose in mg/dl using this equation:[25]

HOMA-IR = (Fasting blood glucose) x (Fasting serum insulin) / 405

The modified- Ferriman-Gallwey (mFG) score is visual grading method used in the clinical assessment of hirsutism. Hirsutism is characterized as a final score of 8 or higher[26]. Global Acne Grading System (GAGS) was used to calculate acne score. The overall severity is the sum of the six regional ratings. Each is determined by multiplying the grade described in (where a comedone counts as 1, a papule as 2, a pustule as 3, and a nodule as 4) by the factor according to place as factor 2 for each one as forehead, right check and left check while 1 for nose and 1for chin, three for upper back and chest. .Patients with Acne score (1 to 18) represent minor acne, (19-30) demonstrate moderate acne score, while from (31-38) show sever score and finally the acne score above 39 represented as very severe cases [27].

Ethical Consideration (No. 1292)

The study was discoursed and accepted by scientific and ethical committee of AL - Mustansiriyah University's pharmacy college and Salah al-Din Governorate, and an agreement was achieved with the Salah al-Din Health Department. After thoroughly explaining the study's objectives and assurances that the data collected was acceptable, the patient's written agreement was obtained.

Statistical Analysis

SPSS version 27 was used for the statistical analysis. Categorical variables were displayed as frequencies and percentages. Continuous variables were displayed as (Means SD). The 3 groups' means were compared using the ANOVA test. The means of 2- paired readings were contrasted using a paired t-test. When a variable was not normally distributed, the Kruskal-Walli's test was applied to compare three groups. When a variable was not normally distributed, the Wilcoxon Signed Ranks Test was employed to compare two paired readings. The correlation between categorical variables was estimated using the Fisher's Exact Test and Pearson chi-square. Significant data was defined as a p-value of lower than 0.05.

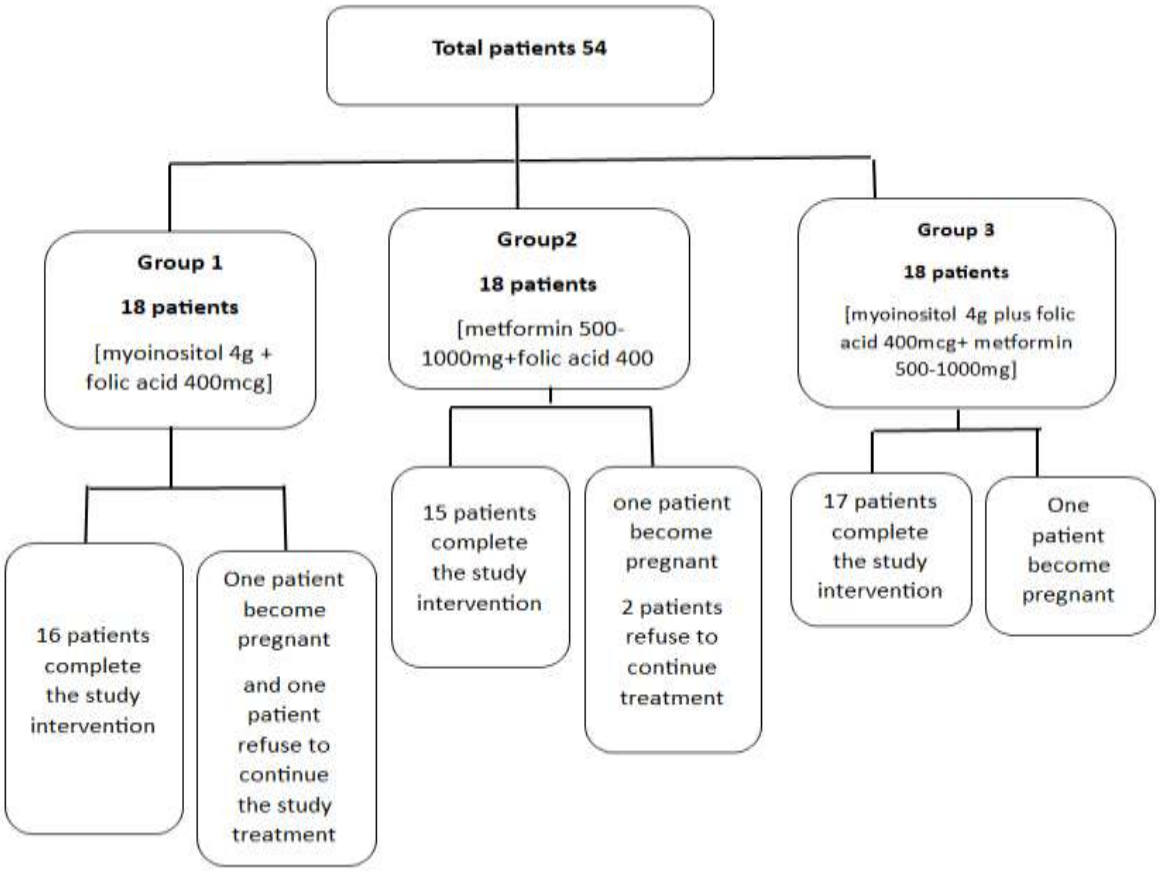

Figure 1. The flow chart of study groups.

Results

1.PCOS Patient Demographics and Disease Features

The disease features and patient demographics of 54 female patients, including 18 patients with each group. All patients ranged in age from 18 to 40 years, and the mean age for the group 1 patients was 24.39± 5.48 years, 28.11 ± 6.02 years for group 2 patients, and 32.44 ± 5.62 years for group 3 patients. Among PCOS women, 30 patients (55.6%) were married and 24 patients (44.4%) were single distributed between the 3 groups.

A positive PCOS family history was seen in (33.3%) patient group 1, (55.6%) group 2 patients, and (44.4%) among patients in group 3. A large number of women, 33 (61.1%), had a duration of symptoms of 2-10 years. Concerning BMI, group 1 presented with an average weight of (24.46 ± 2.88) kg/m^2, while patients in groups 2 and 3 were obese of classes I and II with means of (33.01 ± 4.79) and (35.08 ± 7.55) respectively.

Table 1. Patient demographics and PCOS disease features (N=54)

Study variables	Study groups			Total (N=54)	P-value
	Group 1 (N=18)	Group 2 (N=18)	Group 3 (N=18)		
Age (years)	24.39 ± 5.48 (18-35)	28.11 ± 6.02 (18-40)	32.44 ± 5.62 (22-40)	28.31 ± 6.51 (22-40)	**<0.01****
Marital status Married Single Total	6 (33.3) 12 (66.7) 18 (100.0)	8 (44.4) 10 (55.6) 18 (100.0)	16 (88.9) 2 (11.1) 18 (100.0)	30 (55.6) 24 (44.4) 54 (100.0)	**0.002***
Residence Urban Rural Total	14 (77.8) 4 (22.2) 18 (100.0)	14 (77.8) 4 (22.2) 18 (100.0)	12 (66.7) 6 (33.3) 18 (100.0)	40 (74.1) 14 (25.9) 54 (100.0)	0.792^{NS}
Family History Positive Negative Total	6 (33.3) 12 (66.7) 18 (100.0)	10 (55.6) 8 (44.4) 18 (100.0)	8 (44.4) 10 (55.6) 18 (100.0)	24 (44.4) 30 (55.6) 54 (100.0)	0.407^{NS}
High calories diet Positive Negative Total	7 (38.9) 11 (61.1) 18 (100.0)	17 (94.4) 1 (5.6) 18 (100.0)	18 (100.0) 0 (0.0) 18 (100.0)	42 (77.8) 12 (22.2) 54 (100.0)	**<0.01****
Duration of symptoms < 2 years 2-10 years >10 years Total	10 (55.6) 7 (38.8) 1 (5.6) 18 (100.0)	5 (27.8) 12 (66.7) 1 (5.5) 18 (100.0)	4 (22.2) 14 (77.8) 0 (0.0) 18 (100.0)	19 (35.2) 33 (61.1) 2 (3.7) 54 (100.0)	0.094^{NS}

Quantitative data were shown as Mean SD, while qualitative data were displayed as Frequency and Percentage. Number of patients (N), Percentage (%), NS = no statistically significant difference (P > 0.05), = significant difference, and ** = very significant.

Three groups' means were compared using an analysis of variance test. Pearson In order to determine whether or not there was a correlation between the research groups, a Chi-square test was used (marital status, depression and family history). Using a Fisher's exact test, we may see how different groups relate to one another and how other research factors affect those groups.

Effect of study intervention on hormonal levels

A substantial decline in testosterone level in the group 1(-34.9%) and group 3(-34.9%) after three months of therapy (P <0.05) was observed. In the meantime, there was no decrease in the testosterone levels in group 2 patients (p > 0.05) Multiple comparison post-hoc test shows no significant difference between group 1 (p=0.469) and group 3(p=0.433). Hormonal change presented in Table (2). FSH levels in each group did not significantly differ from baseline levels three months after therapy (P>0.05). However, following three months of treatment, substantial reduction in Luteinizing hormone level and the LH/FSH ratio was found among group 3. In addition, a large decline in E2 group 1 level and group two following three months therapy when compared to the level before treatment (P <0.01). Additionally, a significant reduction among patients in group 3 (*P*<0.05). Multiple comparison post-hoc test for E2 show no significant difference among the three study groups. The levels of free testosterone (FT), FSH, LH, the LH: FSH ratio, and estradiol (E2) in the three study groups did not significantly alter after three months of treatment (P > 0.05).

Table 2. Outcome of study intervention hormone levels

variables	Study groups						P-value
	G1		G2		G3		
FT (pg./ ml)	n	**(Mean ± SD)**	n	**(Mean ± SD)**		**(Mean± SD)**	
Pre treatment	18	(0.63 ± 0.42)	18	(0.66 ± 0.31)	18	(0.63 ± 0.36)	0.956
Post treatment	16	(0.41 ± 0.26)	15	(0.48 ± 0.33)	17	(0.41 ± 0.19)	0.685
P-value	**0.013***		0.108^{NS}		**0.002***		
% Of change	-34.9%		-27.3%		-34.9%		
Luteinizing hormone (m IU/ml)	n	**(Mean ± SD)**	n	**(Mean ± SD)**	n	**(Mean ± SD)**	
Pre- treatment	18	(9.25 ± 5.29)	18	(10.88 ± 6.29)	18	(11.79 ± 7.23)	0.479
Post treatment	16	(8.28 ± 2.69)	15	(8.20 ± 2.48)	17	(6.42 ± 2.17)	0.058
P-value	0.272^{NS}		0.213^{NS}		**0.003***		
% Of change	-10.5%		-24.6%		-45.5%		
FSH (mIU/ ml)	n	**(Mean ± SD)**	n	**(Mean ± SD)**	n	**(Mean ± SD)**	
Pretreatment	18	(6.34 ± 2.84)	18	(7.21 ± 2.78)	18	(6.50 ± 2.31)	0.58
Post treatment	16	(6.14 ± 1.98)	15	(6.73 ± 3.12)	17	(5.50 ± 1.46)	0.316
P-value	0.578^{NS}		0.529^{NS}		0.083^{NS}		
% Of change	-3.2%		-6.7%		-15.4%		
LH to FSH ratio	n	**(Mean ± SD)**	n	**(Mean ± SD)**	n	**(Mean ± SD)**	
Pre -treatment	18	(1.54 ± 0.82)	18	(1.56 ± 0.96)	18	(1.79 ± 0.79)	0.63
Post treatment	16	(1.45 ± 0.58)	15	(1.48 ± 0.92)	17	(1.17 ± 0.23)	0.293
P-value	0.53^{NS}		0.992^{NS}		**0.004***		
% Of change	-5.8%		-5.1%		-34.6%		
E2 (pg./ml)	n	**(Mean ± SD)**	n	**(Mean ± SD)**	n	**(Mean ± SD)**	
Pre treatment	18	(40.94 ± 37.76)	18	(32.99 ± 21.16)	18	(38.19 ± 33.50)	0.747
Post treatment	16	(25.96 ± 27.59)	15	(18.27 ± 10.71)	17	(25.27 ± 26.31)	0.597
P value	**0.001 ** **		**0.001** **		**0.046***		
% Of change	-36.5%		-44.6%		-33.8%		

Data are displayed as mean SD, number of patients (N) , while * (P<0.05) is deemed to be a Significant Difference. Highly significant is estimated to be **(P0.01). Three groups' means were compared using the ANOVA test (pre and post treatment). The means of the two paired readings were compared using the paired t-test (for each group). post-hoc analysis to identify the difference between which groups .FT mean free testosterone, FSH mean follicle stimulating hormone, E2 mean estradiol.

Effect of study intervention on metabolic and obesity markers

Table 3. shows no changes in FBG levels were noticed within each the study group 1,2, and 3 post treatments as compared to pre-treatment (*P*>0.05). The decrease in the FSI level following 3-months of therapy PCOS patient in group one (p=0.02) and group three(p=0.012**)** were significant (*P*<0.05). Multiple comparison post-hoc test for fasting serum insulin between group 1 and group 3 shows significant change in group 1 (p=0.005) while in group 3 (p=0.270).

At baseline and the end of the study period, there was a significant mean difference in HOMA-IR among the three groups, pre- and post-treatment (*P value* <0.05). In groups 1 and 3, there were substantial mean reductions of HOMA- IR three months after starting therapy (*p*=0.025) and (p=0.019) respectively. Multiple comparison post-hoc test for HOMA- IR between group 1 and group 3 shows a substantial change in group 1 (p=0.004) while in group 3 (p=0.365).

{compared equal number of patients within the same group pre- and post-treatment}

In relation to obesity markers, there was a significant decrease of BMI within each group after treatment presented as (-1.6%) group 1, (-3.5%) group 2 and (-2.3%) group 3 (p <0.01). Multiple comparison for BMI (Kg/m^2) post treatment post hoc test between group 2 and group 3 shows a significant difference (p<0.001), while comparison of group 1 to group 3 shows group 1(p<0.001) and group 3 (p=0.208). Also, comparison of group 1 and 2 shows group 1 with significant change (p<0.001) while group 2 with p value equal 0.208. There was a highly significant difference of waist circumference at baseline and after treatment in the three groups (P<0.0١). In addition, there were considerable

decrease within each group before and post- 3 months of treatment which represented as group 1 (-2.5%), group 2 (-2.7%) and (-4.1%) for group 3 ($p < 0.01$). {Equal number of patients were compared within the same group pre- and post-treatment}.

Table 3. Effect of study intervention on metabolic and obesity marker

variables	Study groups						P-value
	G1		G2		G3		
FBG (mg./dl)	n	**(Mean ± SD)**	n	**(Mean ± SD)**	n	**(Mean ± SD)**	
Pre treatment	18	(91.89 ± 6.81)	18	(96.78 ± 8.54)	18	(98.50 ± 9.38)	0.055^{NS}
Post treatment	16	(91.94 ± 5.08)	15	(97.67 ± 5.43)	17	(99.59 ± 10.74)	**0.018***
P-value	0.568^{NS}		0.905^{NS}		0.815^{NS}		
% Of change	0.1%		0.9%		1.1%		
FSI (µIU /L)	n	**(Mean± SD)**	n	**(Mean ± SD)**	n	**(Mean ± SD)**	
Pre- treatment	18	(6.49 ± 2.21)	18	(9.94 ± 4.02)	18	(10.91 ± 6.97)	**0.021***
Post treatment	16	(5.37 ± 1.20)	15	(9.55 ± 4.65)	17	(8.01 ± 4.75)	**0.015***
P-value	**0.02***		0.642^{NS}		**0.012***		
% Of change	-17.3%		-3.9%		-26.6%		
HOMA-IR	n	**(Mean± SD)**	n	**(Mean ± SD)**	n	**(Mean ± SD)**	
Pretreatment	18	(1.49 ± 0.56)	18	(2.51 ± 1.27)	18	(2.46 ± 1.66)	**0.028***
Post treatment	16	(1.21 ± 0.27)	15	(2.30 ± 1.15)	17	(1.98 ± 1.26)	**0.012***
P-value	**0.025***		0.608^{NS}		**0.019***		
% Of change	-19.8%		-8.4%		-19.5%		
Body mass index (Kg/m^2)	n	**(Mean± SD)**	n	**(Mean ± SD)**	n	**(Mean ± SD)**	
Pre treatment	18	(24.46±2.88)	18	(33.01 ± 4.79)	18	(35.08 ± 7.55)	**<0.01****
Post treatment	16	(24.07±2.91)	15	(31.87 ± 4.62)	17	(34.26 ± 7.24)	**<0.01****
P-value	**0.007***		**<0.01****		**<0.01****		
% Of change	-1.6%		-3.5%		-2.3%		
Waist (cm)	n	**(Mean ±SD)**	n	**(Mean ± SD)**	n	**(Mean ± SD)**	
Pre- treatment	18	(81.50 ± 8.08)	18	(101.72± 12.16)	18	(104.61±15.20)	**<0.01****
Post treatment	16	(79.44 ± 7.81)	15	(98.93± 11.96)	17	(100.35±14.53)	**<0.01****
P-value	**0.007***		**<0.01****		**<0.01****		
% Of change	-2.5%		-2.7%		-4.1%		

Data are displayed as mean SD, number of patients (n) NS stands for No Significant Differences (P>0.05), while * (P0.05) is deemed to be a Significant Difference. The means of the three groups were compared using the ANOVA test (pre- and post- treatment). The means of the two paired readings were calculated using the paired t-test (for each group). post-hoc analysis to identify the difference between which groups.FBG mean fasting blood glucose, FSI mean fasting serum insulin, HOMA-IR mean homeostatic model assessment of insulin resistance.

4.Effect of study intervention on Serum GPX and HsCRP Levels

There was a substantial increase in GPX in the three groups (42.9%) in group 1, (45.3%) in group 2, and (53.8%) in group 3 when compared to the pre-treatment level after three months of treatment (***P*** <0.05). Multiple comparison for GPX post- treatment according to study of group2 with group 3 shows group 3 with significant change (p=0.02) while group 2(p=0.515). Also, the comparison of group1 with group 2 present with significant change in group 1(p=0.02) in relation to group 2(p=0.101).

There was a significant difference in (HsC_RP) among the three groups' pretreatment (P <0.05). Also, similar substantial variations were observed following three months of treatment (**p** < 0.05).

After treatment, no important change was noticed in each of the three groups ($p > 0.05$) the changes presented in Table (4). {compared equal number of patients were within the same group pre- and post-treatment}.

Table 4. Effect of study intervention on Serum GPX and HsCRP Levels.

variables	Study groups						P-value
	G1		G2		G3		
GPX (Pmol/ml)	N	**(Mean ± SD)**	N	**(Mean ± SD)**	N	**(Mean ± SD)**	
Pre- treatment	18	(17.59 ± 8.67)	18	(16.46 ± 8.86)	18	(24.48 ± 12.68)	0.1^{NS}
Post treatment	16	(30.78 ±12.60)	15	(36.82 ± 24.92)	17	(53.00 ± 33.86)	0.059^{NS}
P-value	**0.005***		**0.04***		**0.02***		
% Of change	42.9%		45.3%		53.8%		
HsCRP (mg./L)	n	**(Mean ± SD)**	n	**(Mean ± SD)**	n	**(Mean ± SD)**	
Pre treatment	18	(2.29 ± 2.38)	18	(7.74 ± 9.67)	18	(11.70 ± 14.68)	**<0.01 ****
Post treatment	16	(2.53 ± 2.03)	15	(8.49 ± 10.85)	17	(10.79 ± 11.71)	**0.005***
P-value	0.57^{NS}		0.798^{NS}		0.177^{NS}		
% Of change	9.5%		8.8%		-7.8%		

Data existing as (Mean ± SD), (N) mean patients number, * P ≤ 0.05 was significant. NS stands for No Significant Differences, while ** (P0.01) is regarded as a highly significant difference. Three groups' means were compared using the ANOVA test (pre and post treatment). Using the paired t-test, to determine the means of the two paired readings (for each group). post-hoc analysis to identify the difference between which groups .GPX mean glutathione peroxidase, HsCRP mean high sensitivity C-reactive protein.

Effect of study intervention on Hirsutism score and Acne score

At baseline, there was no significant difference in hirsutism score among study groups 1, 2, & 3 (P > 0.05). There was a substantial mean reduction of hirsutism score after three months of therapy in groups 3 and 2 (p < 0.05) but not in group 1(-9.6%, -5.6%, and -2.4%), respectively. Multiple comparison post-hoc test for hirsutism score after treatment between group 2 and group 3 presented as (p=0.169) and (p=0.607) respectively.

Among group 1, 2, and 3 patients, there was no significant difference in the acne grade before and after treatment (P>0.05). A substantial difference in Acne grade within groups 2 and 3 presented as (-26.6% and -24.1%) respectively, after three months of treatment (*P value* <0.05).

Multiple comparison post-hoc test for Acne score after treatment between group 2 and group 3 presented as (p=0.974) for group 2 and (p=0.858) for group 3. An important change in Acne grade observed in group 3 as there was a changed in acne scores from moderate to mild in four patients, while the remaining patients still had a mild degree {Equal number of patients were compared within the same group pre- and post-treatment}.

Table 5. Effect of study intervention on Hirsutism score and Acne score

variables	Study groups						P-value
	G1		G2		G3		
Hirsutism score	n	(Mean ± SD)	n	**(Mean ± SD)**	n	**(Mean ± SD)**	
Pre- treatment	18	(10.56 ±6.20)	18	(13.06 ± 4.30)	18	(10.61 ±2.75)	0.192^{NS}
Post treatment	16	(10.31 ± 5.61)	15	(12.33 ± 3.56)	17	(9.59 ± 2.21)	0.152^{NS}
P-value	0.07		**0.027***		**<0.001***		
% Of change	-2.4%		-5.6%		-9.6%		
Acne score	n	**(Mean ± SD)**	n	**(Mean ± SD)**	n	**(Mean ± SD)**	
Pre treatment	18	(6.50 ± 5.06)	18	(7.33 ± 6.02)	18	(7.61 ± 7.17)	0.853
Post treatment	16	(5.44 ± 5.07)	15	(5.38 ± 5.62)	17	(5.78 ± 5.72)	0.973
P-- value	0.292		**<0.001***		**0.003***		
% Of change	-16.3%		-26.6%		-24.1%		

The data were described as (Mean SD). (N) the mean number of patients, and * P 0.05 was considered significant. No Significant Differences (NS) (P>0.05); Highly Significant Differences (**) (P0.01). The ANOVA test was used to compare the means of the three groups (pre- and post- treatment). post-hoc analysis to identify the difference between which groups. The means of two paired readings were compared using a paired t-test (for each group)

Discussion

The polycystic ovarian syndrome is a hyperandrogenic woman's condition linked to several serious health issues[28].The principle of PCOS treatment involves treating the syndrome's essential features, including hyperandrogenism and insulin resistance, using insulin-sensitizing drugs.

In the current research, patients with a positive family history approximately (44.4%) of all study group patients. This outcome is in line with Tehrani *et al.*, who reported that (45%) of PCOS patients had a positive history in their families [29]. These finding confirm that PCOS have significant genetic predisposition as among first-degree female relatives the prevalence of PCOS has increased by 5 to 6 times [4].

Also, one of the most significant characteristics of PCOS is obesity that affects between 61 and 76% of sick women[30]**.** The current finding presented that the majority (74%) of PCOS patients were overweight or obese, whereas just (25.9%) were considered to be of average weight. Previous study by Pradhan *et al*. also stated that more than half of participants were obese[31].

As a result of insulin resistance and hyperandrogenaemia which modify lipolysis and lipogenesis by altering adipocyte function and distribution and consequently lead to central obesity which characterized by expanded waist circumference[4].

The current study demonstrates all PCOS participants in the research groups had elevated free testosterone levels at baseline as the reference range of free testosterone is 0.1-0.9 ng/mL [32]. Following three months of therapy, a dramatic reduction in testosterone level in PCOS patients received Myoinositol (-34.9%) and those received the combination of Myoinositol and Metformin (-34.9%) (P<0.05), but not on Metformin alone. This finding was consistent with that of clinical study on 70 patients to compare the effects of 16-week treatment with Myoinositol and metformin revealed that free testosterone was decreased more with Myoinositol compared to Metformin (-0.10 ± 0.10) and (-0.08 ± 0.08) respectively [20]. Similar findings from open labelled, randomized, comparative clinical study conducted in 2017 on 60 patients found that the Myoinositol decreased total testosterone by 6.46 meanwhile metformin increased testosterone after 24 weeks by 6.97[19]**.** Collectively, seven randomized controlled trials were included in meta-analysis by Unfer *et al***.** (2017), reported controversial outcome of Myoinositol on serum testosterone. However, compared to other therapies, Myoinositol supplementation for up to 24 weeks showed a considerable rise in Sex hormone binding globulin (SHBG) levels[17]. Hyperinsulinemia also causes an increase in GnRH pulse frequency, though the increase in LH dominates the follicle-stimulating hormone (FSH) rise. This results in an increase in the production of ovarian androgen, an increase in follicular maturation, and a decrease in SHBG, all of which help PCOS advance[33].

The current study revealed comparable decrease in both LH and FSH following 3 months of myo-inositol and metformin monotherapy, though marked decrease was produced when both drugs were combined particularly on LH level (p<0.01). Accordingly, compared to pre-treatment levels in the current investigation, the LH: FSH ratio was considerably lower following a combination of myoinositol and metformin medication (P<0.05). This effect occurs due to a decline in LH level rather than a rise in FSH. The decrease in LH may be caused by a decrease in androgen, which prevents inappropriate androgen feedback on the pituitary and hypothalamus [34]. Despite a new consensus opposing its usage, PCOS is usually diagnosed using LH/FSH ratio, which stands for luteinizing hormone to follicle-stimulating hormone. LH to FSH ratios are usually normal in healthy women vary from 1:2, however in PCOS patients, this ratio is reversible and can rise to 2 or 3 levels due to LH levels greatly elevated [35].

The results of a recent systematic review - meta-analysis by Azizi *et al.* (2021) stated the reduction of the LH after metformin treatment was substantially more efficient than that of the myoinositol group (p<0.001)[36].Meanwhile, Anupama *et al*. (2021)**,** in a prospective, randomized controlled trial on 72 patients, reported significant change in the ratio of luteinizing hormone to follicle-stimulating hormone (LH/FSH) among PCOS patients received metformin 1g/d alone or with MI 550 mg 2 times per day orally for six months (p=0.007) [37].

Many other conflicting results were reported previously, a 3-arm prospective randomized clinical study like our study in comparing myoinositol and metformin in PCOS, stated an important change in LH:FSH ratio after myoinositol therapy, but not with metformin group and Myoinositol + metformin treatment [21].On the other hand, Nabi *et al.(*2020) found that the LH/FSH ratio changed significantly(-0.17 ± 0.18) after Myoinositol and (-0.20 ± 0.26) after metformin (p-value <0.001)[20]. Also**,** Nehra *et al*. (2017) found that LH/FSH ratio dropped in both myoinositol and metformin groups; in myoinositol, they fell by 0.48 and 0.60, respectively [19].

The most powerful circulating estrogen is estradiol (E2) in healthy premenopausal women, and the majority of E2 is produced by aromatase activity in granulosa cells of the ovary by thecal T conversion[38].The previous study by Faraj *et al.* [39] found that E2 in healthy control women was 42.15±13.20 (pg./ml) and increase in the PCOS women 97.66±22.36 (pg./ml)[39]. Invers finding from the current study is that most PCOS patients have low E2 levels at baseline and three months after study intervention, a significant decline was seen in all study groups(P<0.05).

In the current study, the baseline level of metabolic parameters (glycemic status and body weight) presented some difference among study groups despite of strict randomization, which is not

observed in other laboratory investigations. The glycemic status after 3 months of treatment revealed mild improvement in FBG following each study interventions, this is probably due to short duration of treatment for a newly diagnosed PCOS patient. Nevertheless, the current finding showed significant improvement in FSI level among PCOS patients received Myoinositol monotherapy and more effect when combined with metformin (P<0.05), but no effect with metformin alone. This finding is attributed to the fact that Insulin resistance was best resolved by using two insulin-sensitizing agents. Accordingly, it was expected to find a significant decrease in IR expressed by HOMA IR score after 3 months of treatment with Myoinositol alone and the combined therapy equally (-19.8% vs -19.5%) respectively, (P<0.05).The decrease in FSI in the current study after myoinositol and metformin was consistent with Chirania *et al.*[21].In contrast, Nas *et al.*,observed a recognizable, but not significant changes in FSI after 6 month treatment with metformin and Myoinositol treatment [22]. The current study presented with early insulin resistance around (2.5) with (42.6 %) of patients present with insulin resistance. However, recent research suggests that some abnormal insulin action may be caused by inositol depletion, which can cause insulin resistance, as well as inositol phosphoglycan (IPG) is regarded as mediators of insulin action[19], which explains the reason the use of myoinositol monotherapy in the current finding had a significant effect in reducing insulin resistance. According to clinical research by Nehra *et al*., after receiving high doses of myo-inositol (2 g/d) and metformin (1.55 g/d) for 24 weeks, the HOMA-IR values in the myo-inositol group decreased by 1.30 and 1.39, respectively, in comparison to the baseline[19]. Another randomised controlled trial found that myoinositol therapy reduced the HOMA index from 4.21 to 3.634 to 4.32 to 4.61, but metformin treatment had no detectable effect[18].

Sex hormone-binding globulin (SHBG) levels are negatively correlated with higher BMI values, but free androgen index (FAI), total testosterone, free testosterone, homeostatic model assessment for insulin resistance (HOMA-IR), fasting insulin, and fasting glucose are positively correlated [41].

In the current study, the majority of PCOS patients were obese and overweight, after 3 months of treatment, a significant decrease in both BMI and WC were obtained among all patients even when they used the Myoinositol alone (*P* <0.01), more effect was obtained when combined with metformin. This was in consistent with a study where, the effects of 16-week treatment with high doses of two insulin-lowering therapies myoinositol 2g/d and metformin 1g/d, the BMI was significantly decrease in both groups (22.26 ±2.71 to 20.82±2.73) and (23.04±2.94 to 21.35±2.70) respectively in PCOS women[20], despite their BMI were near normal. More similar findings has been reported in further randomised controlled trials[18].

Insulin resistance has been linked to reactive oxygen species and oxidative status. OS decreases the pancreatic cells' ability to secrete insulin and directly stimulate hyperandrogenism in response to hyperglycemia [42]. As a result, treatment with an insulin-sensitizing agent ensures a decrease or elimination of oxidative stress status. oxidative status is now considered key player in pathogenesis of PCOS associated with IR[43], accordingly, the antioxidant effect of Myoinositol expected to produce additional benefits in controlling the oxidative status related to the syndrome. The findings of current study revealed that all PCOS patients existing with a low level of glutathione peroxidase (GPX), an endogenous antioxidant marker related to reactive oxygen species (ROS).

Accordingly, treatment with an insulin-sensitizing agent ensures a decrease or elimination of oxidative stress status. This effect was produced by the current study intervention, where all PCOS patients obtained improvement in the level of GPX level significantly after all types of treatment particularly with Myoinositol, (P<0.01). A controlled clinical study on 50 PCOS women and 50 healthy, found that when compared to controls (54.32 1.51), PCOS dramatically raised the activity of the erythrocyte antioxidant enzymes GRX (63.42 2.78)[44].

Furthermore, the current study found the inflammatory markers hsCRP was above 2 mg/L among all PCOS patient with no significant change after either intervention. Similarly high level of inflammatory status among PCOS women as reported by AL-Watify *et al*., which was (8.72 ± 1.4) mg/L compared to (3.4 ± 2.12) mg/L for Control women [45]. Also, in a cross-sectional study on 66 PCOS patients, found that the average hs-CRP level was (2.41.1 mg/L), which was above the 2 mg/L cutoff and indicated a modest level of inflammation [31].

Concerning excessive hair growth which is primarily caused by hyperandrogenism, compensatory hyperinsulinemia, and insulin resistance which cause a reduction in the sex hormone-binding globulin (SHBG) so causes elevation of free physiologically active testosterone, and ultimately result in hirsutism [46]. As mentioned earlier, hirsutism is described as modified Ferriman Gallwey score (mFG) of 8 or greater. In current study, PCOS patient presented with greater than 8 m FG score at baseline, thereafter marked decrease was noticed in hirsutism particularly after myoinositol and metformin combination therapy, compared to metformin monotherapy (- 9.6%, - 5.6%) respectively, (*P*<0.01). As opposed to the result observed with Chirania *et al.*, as myoinositol showed a

considerable reduction of hirsutism score[21].The effect of myo-inositol was comparable to metformin in previous studies, where both groups showed statistically significant reductions in the mean modified Ferriman Gallwey score (mFG) of hirsutism and acne, but no one was superior[18], and out of the 73% of people with hirsutism, 37% had significantly less hair growth. However, there was just a 10% reduction in facial acne [47].

The finding of the current research presented obvious decline in acne score parallel to the effect on hirsutism particularly after myoinositol and metformin combination therapy, compared to metformin monotherapy this result due to restoration of hormonal imbalance as hormonal imbalances significantly influence acne formation (Androgens, estrogens, insulin, and insulin-like growth factor-1 abnormalities are the primary hormones associated with acne etiology)[48] .

Study limitation

The study's limitation includes the small clinical trial and due to the specificity and accessibility of the test at the time of sampling, there were some gaps in the post-treatment data among the 49 potential patients, particularly in the hormonal test. The glutathione peroxidase kit was unavailable in private laboratories for a few weeks. Additionally, further studies are needed to determine how the addition of myoinositol to metformin affects PCOS patients' metabolic and hormonal profiles.; as a result, erroneous FSH and LH tests may impact the outcomes.

Conclusions

In conclusion, combining Myoinositol plus metformin along with lifestyle adherence improves clinical, endocrine, and metabolic parameters, besides oxidative markers in the polycystic ovarian syndrome women. since insulin resistance was the core complaint of this syndrome, combination treatment provides synergistic therapy effect in improving insulin sensitivity which consequentially improves patient clinical and biochemical disturbances. Compared to metformin, Myoinositol has greater patient compliance, is more well-tolerated, and is thought to be the best medication to complement existing standard therapies with significant effect on insulin resistance and androgen levels.

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Conflicts of Interest

Author declares there is no conflict of interest

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Ethics Statements

The study was conducted in accordance with ethical approval from the Ministry of Health - Saladin Health Directorate {Form No. 2021042- Resolution No. 41 -Decision date: 12/28/2021} in addition to patient approval

Author Contribution

Zainab Abdul Hameed Ibrahim/ processing data collection/ Manal Khaled Abdul Rida /manuscript writing and data analysis.

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